bio-protocol Preprint

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Kcr peptide enrichment

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An abbreviated version of this protocol was published in Science Advances in Mar 2020

Global crotonylome reveals CDYL-regulated RPA1 crotonylation in homologous recombination–mediated DNA repair

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Detailed protocol

1. Mix anti-crotonyllysine agarose bead (PTM Bio Inc., hangzhou, china) suspension and aliquot 40 µl 50% bead slurry to 0.6 ml tube.
2. Wash beads with 0.5 ml pre-chilled PBS. Spin down beads at 1000 x g for 1 min at 4°C, and remove the supernatants. Repeat twice.
3. Dissolve 2 mg peptides in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, 0.5% Nonidet P-40, pH 8.0).
4. Remove any possible precipitates in peptide solution by centrifuging at 12,000 x g for 10 min at 4°C;
5. Mix peptide solution with pre-washed antibody conjugated beads. Incubate at 4°C for 4h with gentle shaking.
6. Harvest beads by centrifuging at 1000 x g for 1 min at 4°C.
7. Wash the beads four times with 1 ml of NETN buffer and twice with deionized water.
8. Elute bound peptides with 1% trifluoroacetic acid. Repeat twice and combine all three elutes.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Cheng, Z. and Liang, J. (2020). Kcr peptide enrichment. Bio-protocol Preprint. bio-protocol.org/prep394.
2. Yu, H., Bu, C., Liu, Y., Gong, T., Liu, X., Liu, S., Peng, X., Zhang, W., Peng, Y., Yang, J., He, L., Zhang, Y., Yi, X., Yang, X., Sun, L., Shang, Y., Cheng, Z. and Liang, J.(2020). Global crotonylome reveals CDYL-regulated RPA1 crotonylation in homologous recombination–mediated DNA repair . Science Advances 6(11). DOI: 10.1126/sciadv.aay4697